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The Vaginal Microbiome: IV. The Role of Vaginal Microbiome in Reproduction and in Gynecologic Cancers

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Objective: This series of articles, titled The Vaginal Microbiome (VMB), written on behalf of the International Society for the Study of Vulvovaginal Disease, aims to summarize the recent findings and understanding of the vaginal bacterial microbiota, mainly regarding areas relevant to clinicians specializing in vulvovaginal disorders.

Materials and Methods: A search of PubMed database was performed, using the search terms "vaginal microbiome" with "reproduction," "infertility," "fertility," "miscarriages," "pregnancy" "cervical cancer," "endometrial cancer," and "ovarian cancer." Full article texts were reviewed. Reference lists were screened for additional articles.

Results: The fourth article of this series focuses on 2 distinct areas: the role of VMB in various aspects of human reproduction and, in sharp contrast, the association between the VMB and gynecologic malignancies. Several of the negative pregnancy outcomes have been associated with an altered VMB. Dysbiosis is remarkably linked with poor pregnancy outcomes from preconception to delivery. The associations between the microbiome and gynecologic cancers are described.

Conclusions: The development of the microbiome research, enabled by molecular-based techniques, has dramatically increased the detection of microorganisms and the understanding of bacterial communities that are relevant to maternal-fetal medicine in health and disease, as well as in gynecological malignancies. Proving causation in cancer is difficult because of the complex interactive nature of potential causative factors. Certain elements of the microbiota have been shown to provoke inflammatory reactions, whereas others produce anti-inflammatory reactions; this balance might be impaired with a change in microbial variety.

Key Words: vaginal microbiome, infertility, pregnancy, miscarriages, preterm birth, cervical cancer, human papillomavirus, endometrial cancer, ovarian cancer

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THE VAGINAL MICROBIOME AND REPRODUCTION

Introduction

Bacteria play a critical role in influencing the vaginal environment in terms of biochemical and inflammatory properties. Therefore, the characteristics of the VMB may affect conception and the ability to carry a fetus to term. Considering that hormonal changes are associated with significant changes in vaginal microbiome (VMB, see part I), one might expect changes in VMB during pregnancy, reflecting physiologic changes linked to the elevated levels of estrogen and progesterone. In addition, infection has long been recognized as a risk factor for poor reproductive outcomes.[1] Dysbiosis, and specifically bacterial vaginosis (BV), is associated with various pregnancy complications,[2] including preterm premature rupture of membranes (PPROMs), spontaneous preterm labor, and preterm birth (PTB; see hereinafter), indicating the possible role of VMB in maintenance of normal pregnancy and reproductive outcomes. A better understanding of the dynamics of the VMB during pregnancy, and the possible relations between the VMB and reproduction, may lead to better diagnostic tools and treatments for complications associated with the complex processes of conception, pregnancy, and birth.

Genital Tract Microbiome and Infertility

Infertility, defined as the inability to conceive after 1 year of regular unprotected intercourse, affects 1 in 7 couples.[3] Female factors, responsible for 35%–40% of cases, include hormonal changes, tubal occlusion, uterine pathologies, maternal age, and systemic or genetic diseases. However, in approximately 35% of couples, the causes remain unexplained despite a thorough assessment,[3] defined as "idiopathic." Assisted reproductive techniques (ARTs), including in vitro fertilization (IVF), are being offered based on the diagnosis; however, the percentage of implantation and pregnancy rates per embryo transfer remains low. The challenge of improving these outcomes is still an ongoing one.

The female reproductive tract microbiome has been proposed to affect infertility and the success of ART. Different microbial-associated mechanisms were posited as contributing,[3] including: pelvic inflammatory disease associated with the presence of specific bacteria in the uterus, tubal occlusion, the correlation between certain bacteria and endometriosis,[4] the composition of endometrial microbiome, and the entrance of cervicovaginal bacteria to the upper genital tract with the sperm during fertilization. However, because of the difficulties in obtaining upper reproductive system samples from healthy women, few data are available to date.

Increasing evidence has shown the presence of microorganisms not only in the vagina but also in the uterus, which was previously considered a "sterile niche."[5–7] Lactobacilli are the most represented genus in the endometrium according to some studies[6,8,9] but not all[10]; however, it is estimated that the upper reproductive system hosts 10,000 times fewer bacteria than the vagina.[11] The uterine microbiota has been proposed to be able to

modulate the endometrial cells' functions as well as the local immune system and to prevent uterine infections.

It has been suggested that infertile women host a different microbiota, both in the lower and/or the upper reproductive tract, compared with fertile women.[12–15] Moreno et al.[9] compared the endometrial and VMB of fertile, healthy women and found similar microbiome profiles between the 2 analyzed body sites in 80% of the cases. Campisciano et al.[14] found significant variations of VMB in idiopathic infertile women compared with 3 groups: healthy, BV-affected, and nonidiopathic infertile women, distinguishing the idiopathic infertile women from the others. Wee et al.[13] compared fertile and infertile women and found that *Ureaplasma* and *Gardnerella* were more abundant, respectively, in the vagina and the cervix of the latter.

Amato et al.[16] analyzed the vaginal and seminal microbiome of 23 couples with idiopathic infertility undergoing intrauterine insemination, aiming to correlate microbial features with pregnancy rates. They found that VMB of idiopathic infertile women differed from controls, as well as different patterns of *Lactobacillus* species among idiopathic infertile women, with *L. crispatus* being associated with higher rate of intrauterine insemination success. Therefore, they suggested including VMB evaluation in the work-up of idiopathic infertility, before recommending intrauterine insemination.[16]

In addition, it has been suggested that the outcomes of IVF procedures may be affected by the resident microbiota. While lactobacilli have been reported to exert beneficial effects, endometrial or vaginal dysbiosis has been related to a worse success rate in terms of lower implantation rates, pregnancy rates, ongoing pregnancies, and live birth rates.[9,17]

In summary, current data suggest that microbial dysbiosis is associated with infertility and may also play a limited role in IVF outcomes.[18] The uterine microbiota, because of its low biomass and the difficult sampling, is challenging to be studied. It also seems that the evaluation of the partner's semen microbiota composition is important as it has implications not only on the composition of the female one but also on the reproductive health of the couple and offspring.[3]

Vaginal Microbiome and Pregnancy Outcomes

Accumulating evidence links microorganisms to the etiology of various maternal-fetal conditions, including PTB, PPROM, fetal growth restriction, late abortions, and stillbirth.[1] Preterm birth, defined as birth before 37th gestational weeks, is the leading cause of neonatal morbidity and mortality worldwide, with an estimated 15 million babies throughout the world born preterm, and approximately 1 million of them dying from complications of PTB.[1] Several cohort studies have shown that healthy women with normal pregnancy outcomes generally maintain a VMB dominated by *Lactobacillus* species throughout the entire pregnancy.[19]

How the VMB may promote healthy pregnancy outcomes is still under investigation.[20] However, cultivating bacteria has not revealed the precise etiologies of PTB or other adverse obstetric conditions. As it is estimated that 90% of the microorganisms comprising human microbiomes are uncultivable,[21] the development of DNA sequencing technologies may assist in understanding the relationships between the microbiome and pregnancy outcomes.

The Vaginal Microbiome in Pregnancy

Human pregnancy is characterized by an increase of lactobacilli abundance in the vaginal milieu, as well as stable vaginal bacterial composition and low bacterial diversity.[22]

Hormones probably play a crucial factor in determining the VMB composition during pregnancy. Placental estrogen production in pregnancy significantly increases its levels, stimulating maturation of the vaginal epithelium and promoting additional glycogen production and lactobacilli thrive. α-Amylase from the vaginal mucosa processes glycogen to produce maltose, maltotriose, and maltotetraose, which all contribute to lactobacilli growth.[23]

The shift toward *Lactobacillus* species dominance occurs early in pregnancy and is most dramatically observed in women of African ancestry. Recently, Serrano et al.[24] showed that among women who experienced uncomplicated term birth, the VMB changed during pregnancy, becoming more lactobacilli dominated at the expense of *Gardnerella vaginalis* and other anaerobes. Stratification of the profiles according to ancestry revealed minimal differences in the microbiome profiles of pregnant and nonpregnant women of European ancestry. However, pregnant women of African and Hispanic ancestry showed a higher prevalence of bacterial communities dominated by *Lactobacillus* species than their nonpregnant counterparts. The alpha diversities (the diversity within a sample) of the VMB of pregnant women in all groups were lower than those of their nonpregnant counterparts. These findings are in agreement with previous studies.[25,26]

Together, these observations suggest that the compositions of the VMB of women of African and non-African ancestry respond differently during pregnancy due to complex interactions between human and microbial physiology as well as environmental influences, implying that in the case of a high-diversity VMB, the composition could change during pregnancy, reducing adverse obstetrical risks.

MacIntyre et al.[27] reported that a specific lactobacilli community was not required for a term uncomplicated birth. Inline, the predominant *Lactobacillus* species during pregnancy differs according to various studies[26,28–30]; in the cohort of MacIntyre et al.,[27] the predominant species was *L. crispatus*, but Asian and White women were also colonized by *Lactobacillus jensenii* or *Lactobacillus gasseri*.[27] Others have reported that *Lactobacillus iners* decreased in the second and third trimester compared with the first trimester, whereas *L. crispatus* increased in the second trimester.[28] Nevertheless, in other cohorts, the VMB was mostly dominated by *L. iners*.[26]

There is a complex interplay between the VMB, local immune response, and its metabolic milieu.[31] Lactic acid increases the release of interleukin (IL)-1β and IL-8 from vaginal epithelial cells. This suggests a synergistic association flanked by inflammatory activation in the host and microbial composition. These activities are most probably dependent on mutually intrinsic (genetic) and extrinsic (environmental) factors. It is known that vaginal epithelial cells only produce the L-lactic acid isomer. Instead, lactobacilli and other lactic acid–producing bacteria produce both the D- and the L-lactic acid isomers. Witkin et al.[32] discovered elevated levels of D-lactic acid in vaginas that were colonized almost exclusively by *L. crispatus.* They suggested that an increase in the proportion of D- to L-lactic acid promoted the expression of metalloproteinase inducer in control of activating the matrix MP-8 and consequently modify the integrity of the uterine cervix responsible for cervical ripening.[23,32]

Vaginal Microbiome and Miscarriages

Miscarriages, complicating up to 25% of pregnancies, result from various factors, in half of the cases from chromosomal aberrations. However, evidence supports an infectious etiology as well, with reported histological chorioamnionitis in 77% of miscarriage samples compared with 0% of control cases of induced loss for fetal anomaly.[33] Furthermore, a relation between BV and increased risk of miscarriage was reported.[34]

In a study aimed to characterize vaginal bacterial composition in early pregnancy and its relationship with miscarriages, it was found that first trimester miscarriages were associated with *Lactobacilli* species depletion and a significantly higher proportion of samples dominated by CST IV (see part I).[35] Consistent

with this, bacterial alpha diversity was significantly higher in miscarriage samples than in matched control samples.

The certain mechanism associating first trimester miscarriages with vaginal dysbiosis is still undetermined. When elucidated, it may represent a modifiable target for miscarriage prevention.[35]

Vaginal Microbiome and Preterm Birth

Preterm birth is considered a multietiological phenomenon, and infection is thought to contribute to at least one third of these cases.[19] Preterm birth associated with infection is assumed to be secondary to pathogen ascension from the vagina. This hypothesis is supported by the similarity observed between bacteria found in the placenta and fetal membranes of PTB cases and vaginal microbiota. As a result, studies were conducted aiming to characterize VMB in women with PTB or PPROM. This topic was recently reviewed by Bayar et al.,[19] which concluded that the most consistent finding across almost all studies is the benefit of a vaginal microbiota dominated by *L. crispatus*.

In the United States, women of African ancestry are significantly more likely than women of European ancestry to experience a PTB and PPROM. The difference is presumably attributable to multiple factors, including environmental factors, socioeconomic status, and genetic factors.[24] Nevertheless, the concept that ethnicity is a strong determinant of the VMB and its effect on PTB rates has been extensively studied. In a study including mostly White population, *Lactobacillus*-depleted vaginal CST were correlated with reduced gestational age at delivery, and high abundances of *Gardnerella* or *Ureaplasma* were associated with greater risk for PTB.[36] A subsequent study showed that although *Lactobacillus* species depletion and greater abundance of *Gardnerella* were more common in women of African ancestry, it represented a risk factor for PTB only in White women.[37] As part of the integrative Human Microbiome Project, Fettweis et al.[38] compared the VMB and cytokine profile between women of African ancestry delivering preterm and at term. Analyses showed that the former had significantly lower levels of *L. crispatus*, higher levels of BVAB1, *Sneathia amnii*, TM7-HI, and a group of *Prevtolla* species. Women who delivered at term were more likely to have *L. crispatus* and reduced prevalence of *Atopobium vaginae* and *G. vaginalis*. Preterm birth was also associated with a vaginal cytokine profile richer in proinflammatory cytokines, including eotaxin, IL-1β, IL-6, and MIP-1β. In European studies comprising mostly White women,[19] an increased risk of PTB was associated with *L. iners*, whereas a protective effect for *L. crispatus* dominance was found.[39,40] Other studies showed that vaginal dysbiosis is associated with PPROM and constitutes a risk factor for chorioamnionitis as well as for neonatal sepsis.[19]

Summary

Several of the negative pregnancy outcomes have been associated with an altered vaginal milieu. Dysbiosis is remarkably linked with poor pregnancy outcomes from preconception to delivery. More research is essential to explore the mechanisms causing adverse events and the relationship between the VMB and the immune system. The most consistent finding across almost all studies is the benefit of a vaginal microbiota dominated by *L. crispatus*. Many studies have examined the efficacy of antibiotics for the treatment and prevention of PTB, and almost entirely targeted BV. The failure of antibiotic therapy to improve PTB in the context of abnormal VMB, has led to an interest in the modulation of the VMB using live biotherapeutic products or probiotics (see part V). To date, none of the treatment modalities that were studied showed beneficial effects upon the PTB rate.[19]

THE VAGINAL MICROBIOME AND GYNECOLOGIC CANCERS

Introduction

Microorganisms, including viruses and bacteria, are estimated to have a role in 15% of malignant neoplasms.[41] Oncogenic bacteria and viruses directly modulate carcinogenesis through specific toxins that can damage host DNA or by integration of oncogenes into host genomes, respectively. Nevertheless, clinical and animal studies suggest that carcinogenesis may be driven by global changes in the microbiome, rather than by single pathogens.[42] In this case, the pathophysiology is believed to result from altered host defense responses to dysbiotic microbiota.[43] Dysbiosis may promote epithelial barrier dysfunction, immune dysregulation, genotoxicity, and/or inflammation, collectively creating a tumor permissive microenvironment.[42–44] Interestingly, some bacteria, such as *Chlamydia trachomatis*, induce epithelial-to-mesenchymal transition of infected cells, which might promote loss of epithelial cell adhesion and downregulation of DNA damage responses, inducing carcinogenesis.[45] In addition, bacterial communities may contribute to the etiology, disease severity, and/or response to treatment of gynecological malignancies.[46]

Cervical Cancer, the Vaginal Microbiome, and Human Papillomavirus

Cervical carcinoma (CC) is the most common human papillomavirus (HPV)–related malignancy, with high-risk HPV genotypes, especially HPV-16 and HPV-18, being well-recognized oncogenic factors, detected in 99.7% of the cases.[47] Of high-risk HPV infections, 85%–90% are spontaneously cleared and only 10%–15% persist, consequently causing precancerous cervical intraepithelial neoplasia. These lesions may subsequently progress to invasive CC.[48]

Various factors, including age of sexual debut, parity, usage of contraceptives, smoking, and other STIs, have been shown to increase the risk of progression to cervical neoplasia among HPV-infected women.[49–52] Emerging evidence suggests that the VMB also participates in cervical carcinogenesis.[52–55] Studies reported associations between non-*Lactobacillus*–dominant VMB, HPV infection, and its persistence.[52–55] In addition, BV has been associated with an increased risk of HPV acquisition and decreased clearance.[56]

Three meta-analyses supported a link between a non-*Lactobacillus*–dominant VMB and cervical disease via the effect of the microbiome on HPV acquisition, persistence, and the development of cervical intraepithelial neoplasia (CIN).[57–59] Vaginal microbiome dominated by *L. iners* was also associated with higher odds of HPV and progression to cervical neoplasia compared with *L. crispatus* dominance.[57]

Three microbiome studies have included women with cervical abnormalities and cancer.[53–55] They consistently reported depletion of *Lactobacilli* species and a substantial increase in VMB diversity in women with cervical intraepithelial neoplasia and invasive carcinoma compared with healthy women. Aiming at identifying particular bacteria, which are associated with cervical disease, they pointed out that 3 BV-associated microorganisms: *Sneathia sanguinegens*, *Anaerococcus tetradius*, and *Peptostreptococcus anaerobius* were considerably more abundant among patients with high-grade CIN than in those with low-grade abnormalities.[53] The presence of *Sneathia* in the vagina was suggested as a metagenomic marker for HPV persistence and progression of CIN.[52] Not unexpectedly, a significant increase in vaginal pH, correlated with the depletion of lactobacilli, was related to the severity of cervical disease progression.[55]

The increasing body of evidence implies a complex relationship between the host, local microbiome, and HPV during cervical carcinogenesis. A cross-sectional study evaluated multiple immune

mediators in the cervicovaginal microenvironment in women with CC/CIN and those without neoplasia (HPV infected or not). Nonlactobacilli-dominated VMB was associated with increased proinflammatory and chemotactic mediators,[55] as well as with altered local metabolic profiles,[60] which might directly or indirectly contribute to cervical carcinogenesis.[61]

Endometrial Cancer and Vaginal Microbiome

Environmental factors, including obesity, unopposed estrogen exposure, and inflammation, are considered major risk factors for endometrial cancer (EC) development.[62,63]

Endometrial colonization with bacteria was hypothesized to promote carcinogenesis via microbiota-mediated stimulation of cytokines secretion from the host cells or by mediating dysbiosis-related growth factors.[42,64]

Walther-António et al.[65] studied the microbiome in patients with endometrial hyperplasia, EC, and benign uterine conditions, using samples that were taken from various sites along the reproductive tract. The microbiome sequencing revealed that the microbiomes of the vagina, cervix, fallopian tubes, and ovaries are significantly correlated. There was a structural microbiome shift in the EC and hyperplasia cases, distinguishable from benign cases. In samples belonging to the EC cohort, several taxa were found to be significantly increased, including *A. vaginae* and *Porphyromonas* species, which were found to be associated with disease status, especially if combined with a vaginal pH >4.5. The authors suggested that a possible subclinical vaginal infection might cause chronic upper genital tract infection and inflammation that may trigger carcinogenesis.[66]

Another hypothesis correlates the associations between intestinal and VMBs as related with EC.[46] Gut microbiome composition can affect the risk of EC directly by increasing adiposity, with a resultant increase in circulating estrogens, or by indirectly governing the metabolism of estrogens.[61,66,67]

The gut microbiota can indirectly influence the genital microbiota composition through estrogen-mediated mechanisms.[52,67,68] Estrogen facilitates the growth of lactobacilli through induction of glycogen production in epithelial cells; however, it was shown that circulating estrogen levels are influenced by gut microbiota,[69] leading to a concept of an estrogen-mediated gut-vagina axis.[67] The relationship between these 2 mucosal sites involves enteric bacteria that metabolize estrogens; the collection of these microorganisms and their genes was termed the "estrobolome."[68] These microorganisms secrete enzymes (β-glucuronidase and β-glucosidase), which deconjugate hepatically conjugated estrogens, resulting in free estrogen, which is absorbed to the circulation,[69] subsequently transported to distal sites, where it binds to its receptors and produces its physiological functions. Thus, an alteration of the gut microbiota diversity, which may result in a lack of estrogen-metabolizing bacteria could influence the VMB composition via the estrobolome. Similarly, a dysbiotic gut microbiome that increases estrogens deconjugation will be followed by increased estrogen levels and hyperestrogenic environment, which in turn increase the risk of estrogen-dependent tumors, including EC.[70]

Microbiota in Tubal and Ovarian Cancer

Genital dysbiosis has been associated with the development of ovarian carcinoma (OC).[71,72] Two cross-sectional studies have compared ovarian tissues in women with OC and healthy women and found a distinct ovarian microbiome in women with OC.[71,72] Specifically, the presence of potentially pathogenic intracellular microorganisms, such as *Brucella*, *Mycoplasma*, and *Chlamydia* species, was found in 60%–76% of ovarian tumors.[72–74]

Other pathogenic microorganisms that were identified in OC tissues included HPV, cytomegalovirus, and *C. trachomatis.*[75] Moreover, the microbiome of the malignant ovarian tissue had distinct microbial signatures compared with the healthy surrounding ovarian tissues within the same individuals.[72] The authors concluded that OC tissue has a significantly different microbiome composition compared with that of surrounding tissue and controls.[72]

Although these studies suggested a link with inflammation, the link between microbiota and ovarian cancer remains unclear. These microorganisms might induce carcinogenesis through direct or indirect mechanisms; however, it is also possible that the tumor microenvironment might also favor the recruitment and growth of anaerobic microorganisms.

The fallopian tubes and ovaries are low-abundancy sites, which makes sample collection without cross-contamination and interpretation of the results challenging.[52] Infertility is considered an independent risk factor for OC. The causes of female infertility are variable, and 14% of the overall causes of infertility are linked to tubal factors. These are usually resulting from salpingitis caused by a previous infection, such as pelvic inflammatory disease.[66] Infections, leading to infertility caused by "tubal factors," may also cause subclinical and possibly persistent microbial infection. With recent advances in the understanding of OC, it is now thought that the fimbriae portion of the fallopian tube is the origin of OC. Subclinical microbial infections within the fallopian tube may cause both infertility and OC.[66]

Summary

The associations between the microbiome and gynecologic cancers are largely unknown. Proving causation in cancer is difficult because of the complex interactive nature of potential causative factors. These difficulties can be overcome by conducting large, international, longitudinal cohort studies.[76] The female genital tract may be more susceptible to microbial insults because it is exposed to the external environment and is populated with numerous bacterial species, like the gut. Certain elements of the microbiota have been shown to provoke inflammatory reactions, whereas others produce anti-inflammatory reactions; this balance might be impaired with a change in microbial variety.

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